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STUDIES IN NON-SPECIFIC COMPLEMENT FIXATION *

V. THE EFFECT OF HEAT ON NORMAL RABBIT AND DOG SERA IN RELATION TO ANTILYTIC AND NON-SPECIFIC COMPLEMENT-FIXATION REACTIONS

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Having considered in previous articles[1] the property of normal rabbit and dog sera of yielding non-specific complement-fixation with various lipoidal and bacterial antigens, and the influence of anesthetics and splenectomy[2] upon this property, and the relation of serum lipoids to the process,[3] in the present article we shall study further the nature and mechanism of the process, and the influence of certain factors upon it, and we shall consider methods for lessening or abolishing its effects in complement-fixation tests with these sera for specific immune bodies.

ANTIHEMOLYTIC POWER OF BLOOD SERUM IN RELATION TO NON-SPECIFIC COMPLEMENT FIXATION

Of primary importance in this connection is a consideration of the anticomplementary or antihemolytic properties of normal blood serum in relation to non-specific complement fixation on the basis that these substances are principally involved, and that in these reactions with normal rabbit and dog sera we have but a summation of the anticomplementary effects of antigen and some substance in the serum.

It is well known that normal human serum, as well as that of animals, may develop antihemolytic properties within a short time after bleeding, even under sterile conditions. Usually the influence of these antilytic substances may be removed by heating the serum at 55 C. for one-half hour, the antilysins in such case being known as thermolabil antilytic or anticomplementary substances; older sera under sterile conditions, sera containing large amounts of dissolved hemoglobin, or comparatively fresh sera infected with various micro-

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[1] Jour. Infect. Dis., 1916, 18, pp. 20, 27.

[2] Ibid., p. 32.

[3] Ibid., p. 46.

organisms, may develop antilytic properties that cannot be removed by this process of heating, and to these substances has been applied the term thermostabil anticomplementary substances. Both these substances are probably of the same general structure and nature, and their effects quantitative rather than qualitative. They are of the greatest importance, however, in all complement-fixation tests, for a serum containing them is capable of absorbing or fixing varying amounts of hemolytic complement in a non-specific manner with a tissue or bacterial antigen.

While fresh, normal human serum alone may contain practically none of these antilytic substances, and while a lipoidal extract may be used as antigen in an amount 10 or 20 times less than its antilytic or anticomplementary dose, yet a combination of serum and extract in a complement-fixation test may show appreciable, or even well-marked, degrees of complement fixation in from 1 to 7% of sera. Noguchi has studied these reactions with particular care and to them has given the name of proteotropic, or false, complement-fixations.

These proteotropic reactions are non-specific, in that they may occur with perfectly fresh serum of a normal individual and with various lipoidal extracts as antigens. Noguchi has observed, however, that they are more likely to occur with crude alcoholic extracts of organs as antigen than with purified extracts, as the acetone-insoluble lipoids fraction. Our studies with fresh, normal rabbit serum and dog serum have given us similar results, but in a larger percentage of sera, especially with dog serum. In other words, the occurrence of complement fixation in a mixture of fresh and active, normal rabbit or dog serum with a lipoidal antigen is similar in several respects to the proteotropic, or false, reaction sometimes found with normal human serum. Likewise, our figures show that crude tissue extracts give a higher percentage of non-specific reactions than extracts freed of various extractives, fatty acids, etc., the extract of acetone-insoluble lipoids yielding the lowest percentage of false, or non-specific, reactions, and those of alcoholic extracts of heart and the same re-enforced with cholesterin, the highest percentage of positive reactions.

The remarkable difference in this connection, however, is that, while the antilytic properties of fresh human serum may be removed or greatly lessened by heating at 56 C. for half an hour, with rabbit, dog, and mule sera the reverse is noted, these sera becoming more antilytic when heated, and yielding higher percentages of pseudo-complement-fixation. Human serum may develop antilytic properties as the result of heating, but this action is not in evidence in complement-fixation tests as ordinarily conducted; whereas the important practical significance of the behavior of dog and rabbit sera in complement-fixation tests is noted when these sera are inactivated by heating at 56 C. for one-half hour and used in complement-fixation tests with various and indifferent tissue extracts and bacterial emulsions as antigens. Then well-marked complement fixation occurs, while in those test tubes containing no antigen, as the serum control tubes, hemolysis is complete.

It is now generally accepted that these serum antilysins exert their antilytic activity by some action upon the complements, and hence they are more generally known as anticomplementary substances. Their relation to the substances causing non-specific complement fixation is so intimate that in this work it was necessary to study the subject from both standpoints.

TECHNIC

The complement-fixation tests were conducted with 3 lipoidal extracts, namely, an alcoholic extract of heart re-enforced with cholesterin, an alcoholic extract of syphilitic liver, and an extract of acetone insoluble lipoids from beef heart; also with 3 bacterial antigens of staphylococci, colon bacilli (human), and typhoid bacilli, described in previous papers.

The lipoidal extracts were used in the same doses as in our regular Wassermann reactions, these amounts being 6 to 12 times less than the antihemolytic doses. The bacterial antigens were titrated before each experiment and used in amounts equal to one-quarter of their antihemolytic doses.

Complement was furnished by the mixed sera of 2 or more guinea-pigs and used in a constant dose of 1 c.c. of a 1:20 dilution (0.05 c.c. undiluted serum). Antisheep hemolysin (rabbit) was titrated each day against this constant dose of complement and 1 c.c. of a 2.5% suspension of washed sheep corpuscles, and used in amounts equal to 2 hemolytic units in the antigen titrations and complement-fixation tests. Other hemolysins, as antihuman (rabbit) and antiox (rabbit), were similarly titrated against their respective cells, and used in amounts equal to double their hemolytic units.

When sera were heated at a temperature of 70 C. and higher, they were first diluted with 4 parts of normal salt solution.

Tests for the antihemolytic action of serum were conducted by incubating heated serum and complement for 1 hour, then adding the cells and 2 units of hemolysin, and re-incubating for 1 hour, after which the results were read.

Complement-fixation tests were conducted by incubating antigen, serum, and complement for an hour, then adding the cells and 2 units of hemolysin, and re-incubating for 1 hour, after which the results were read.

The doses of complement and hemolysin employed are very important in relation to the results. The employment of the amount of complement serum mentioned with 1, instead of 2, hemolytic units of hemolysin increases the percentages of antihemolytic and complement-fixation reactions and conversely an excess of either complement or hemolysin or both over the amounts mentioned tends to decrease the percentages of these reactions.

THE RELATION OF HEAT TO THE ANTIHEMOLYTIC AND NON-SPECIFIC COMPLEMENT-FIXATION PROPERTIES OF SERUM

Heat bears a very important relation to the antihemolytic property of serum and its facility for absorbing complement in a non-specific manner. While the phenomenon may occur with perfectly fresh and unheated rabbit and dog sera, it is greatly intensified as the result of heating the sera at a temperature above 60 C., whereas at higher temperatures the property is less in evidence and disappears.

Of considerable importance in this connection are the observations of Camus and Gley,[4] Müller,[5] Ehrlich and Sachs,[6] Sachs,[7] Bordet and Gay,[8] Sachs,[9] Noguchi,[10] and others, showing that human and animal sera develop antihemolytic properties as the result of heating. Ehrlich and Sachs have attributed this action to the production of complementoids, and we have made studies in this phase, to be discussed later in this paper. Noguchi found that the antihemolytic action of most sera appears when the temperature is raised above 50 C., and he extracted an antilytic principle with ether, called "protectin," that was found capable of withstanding temperatures as high as 90 C., and capable of inhibiting serum hemolysis directly by neutralizing complement, and indirectly after absorption by corpuscles by increasing their resistance. Zinsser and Johnson[11] have studied particularly the thermolabil antilysins of human serum which may be removed or rendered inactive by heating serum to 56 C. Karsner and Pearce[12] have noted the antihemolytic action of dog serum.

With the hemolytic system employed we have occasionally met with perfectly fresh dog serum that was antihemolytic in doses of 0.1 to 0.2 c.c., but the percentage is considerably increased by heating the sera. Fresh serum may be antihemolytic and lose this property as a result of heating to 56 C. for one-half hour, but, as previously stated, a more significant change is the acquisition of antihemolytic activity during the process of heating. This is shown in Table 1, where fresh dog serum, free of antihemolytic activity until heated, at 56 C. acquires antihemolytic activity after an exposure of 20 to 30 minutes. Prolonged exposure to an hour and a half does not materially influence the antihemolytic substances as do higher temperatures in shorter intervals of time.

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[4] Compt. rend. Soc. de biol., 1901, 53, p. 732.

[5] Centralbl. f. Bakteriol., 1901, 29, p. 860.

[6] Berl. klin. Wchnschr., 1902, 39, p. 492.

[7] Ibid., p. 216; Centralbl. f. Bakteriol., I, O., 1905, 40, p. 125.

[8] Ann. de l'Inst. Pasteur., 1906, 20, p. 467.

[9] Deutsch. med. Wchnschr., 1905, 31, p. 705.

[10] Jour. Exper. Med., 1906, 8, p. 726.

[11] Ibid., 1911, 13, p. 31.

[12] Ibid., 1912, 16, p. 769.

TABLE 1

The Antihemolytic Action of Dog and Rabbit Sera Heated at 56 C. for Varying Intervals of Time [Dose 0.2 c.c. Two Units of Antisheep (Rabbit) Hemolysin]

Exposure in Minutes	Dog Serum 6	Dog Serum 17	Dog Serum 9	Dog Serum 30	Rabbit Serum 12	Rabbit Serum 28	Rabbit Serum 42
Fresh..........	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Moderate hemolysis (slightly antilytic)
10..............	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
20..............	Complete hemolysis	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
30..............	Moderate hemolysis (slightly antilytic)	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
45..............	Moderate hemolysis (slightly antilytic)	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Moderate hemolysis (slightly antilytic)	Complete hemolysis
60..............	Slight hemolysis (markedly antilytic)	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis	Complete hemolysis
90..............	Slight hemolysis (markedly antilytic)	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis

As shown in Table 2, the antihemolytic action of heated dog serum is most apparent in doses exceeding 0.1 c.c.; with the largest amounts, as 0.8 to 1.0 c.c., sufficient normal or native antisheep hemolysin is contained in the serum to counterbalance a part of the antihemolytic action of the serum, so that hemolysis is again more complete.

As shown in Table 3, the antihemolytic activity of dog serum becomes apparent after the serum has been heated at 55 C.; at a temperature of 70 C. and higher this activity is lost—a result probably, as Noguchi has pointed out, of the liberation from the serum of a second group of principles in themselves hemolytic directly, or indirectly by increasing the power of serum hemolysins.

This antihemolytic activity of heated serum was found when antihuman (rabbit) and antiox (rabbit) hemolysins were employed, and heated dog serum neutralizes the action of fresh serum. This antihemolytic action, therefore, is non-specific, and, as shown by Noguchi, is probably antagonistic to the serum complements.

The antihemolytic action of dog serum is rather variable, as shown in the selected examples in these tables. With the technic described we have occasionally found fresh dog sera in some degree antihemolytic in dosage of 0.1 to 0.2 c.c.; with sera heated at 56 C. for one-half hour the percentage found to be antihemolytic in these amounts is much increased. In complement-fixation tests with dog serum this antihemolytic activity is likely to prove disturbing and should be remembered.

TABLE 2

THE ANTIHEMOLYTIC ACTION OF DOG AND RABBIT SERA HEATED AT 56 C. FOR 30 MINUTES
[TWO UNITS OF ANTISHEEP (RABBIT) HEMOLYSIN]

Amount of Serum in c.c.	Dog 535	Dog 538	Dog 548	Rabbit 65	Rabbit 40	Rabbit 82
0.1	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.2	Complete hemolysis	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.4	Complete hemolysis	None	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.6	Complete hemolysis	None	Moderate hemolysis (slightly antilytic)	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis
0.8	Complete hemolysis	None	Slight hemolysis (markedly antilytic)	Slight hemolysis (markedly antilytic)	Complete hemolysis	Moderate hemolysis (slightly antilytic)
1.0	Complete hemolysis	None	Slight hemolysis (markedly antilytic)	None	Complete hemolysis	Moderate hemolysis (slightly) antilytic)

Rabbit serum is much less likely to show antihemolytic activity. We have never found a perfectly fresh rabbit serum antihemolytic in doses under 0.4 c.c., unless the serum was deeply stained with hemoglobin and had been secured more than 24 hours before being tested.

Likewise, when heated, rabbit serum is much less likely than dog serum to develop antihemolytic properties. As shown in the tables, composed of selected examples from a large number of tests, a serum may develop antihemolytic properties after an exposure of 30 minutes or more at a temperature between 50 and 60 C. or higher for the same interval of time. In complement-fixation tests with fresh rabbit serum, heated at 55 C. for one-half hour and used in amounts of 0.1 to 0.2 c.c. with the hemolytic system described, we have rarely found any interference with hemolysis in the serum control tubes. This observation has been emphasized in our previous papers on complement-fixation studies with rabbit serum.

TABLE 3

THE ANTIHEMOLYTIC ACTION OF DOG AND RABBIT SERA AFTER HEATING AT VARYING DEGREES CENTIGRADE FOR 30 MINUTES [DOSE 0.2 C.C. TWO UNITS OF ANTISHEEP (RABBIT) HEMOLYSIN]

Temperature C.	Dog	Dog	Dog	Dog	Rabbit	Rabbit
Fresh...........	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Moderate hemolysis (slightly hemolytic)
45..............	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
50..............	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
55..............	Complete hemolysis	Complete hemolysis	Moderate hemolysis (slightly hemolytic)	Complete hemolysis	Complete hemolysis	Moderate hemolysis (slightly hemolytic)
60..............	Moderate hemolysis (slightly hemolytic)	Complete hemolysis	Slight hemolysis (markedly antilytic)	Moderate hemolysis (slightly hemolytic)	Complete hemolysis	Complete hemolysis
65..............	Moderate hemolysis (slightly hemolytic)	Complete hemolysis	Slight hemolysis (markedly antilytic)	Complete hemolysis	Complete hemolysis	Complete hemolysis
70..............	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
75..............	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
80..............	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis

In these respects rabbit serum is more like human serum; it is highly probable, however, that the antihemolytic substances so frequently present in dog serum and much less frequently found in rabbit serum are identical and that the differences are quantitative rather than qualitative.

As stated in previous papers, fresh, active, normal dog and rabbit sera occasionally absorb or fix complement with lipoidal and various bacterial antigens. This property is greatly increased by heating, and, as a general rule, develops at a lower temperature and with a shorter exposure than the antihemolytic activity.

The question naturally arises whether apparent complement fixation by normal dog and rabbit sera with various antigens is actually due to the influence of antihemolytic substances of the serum plus the antihemolytic action of the extracts used as antigens. If this were true, the results could be ascribed to the destruction or inactivation of complement rather than to a true absorption of complement, according to our present conception of the phenomenon of complement fixation. Are the antihemolytic and hypothetical complement-fixing bodies developed and destroyed by the same temperature, or may a serum be free of antihemolytic activity and yet yield positive complement-fixation?

As stated in this paper and emphasized in our previous communications on non-specific complement fixation by normal rabbit and dog sera, the phenomenon of complement fixation was studied with the same technic as employed in our regular Wassermann tests, in which the antihemolytic action of each serum was always controlled in the serum control tube containing the maximal dose of serum and the antigens were always carefully titrated and used in amounts at least from 4 to 12 times less than their antihemolytic doses. With ready and complete hemolysis of the serum and antigen controls, showing that the antihemolytic substances of the sera and antigens were not appreciably in evidence, with complete or well-marked inhibition of hemolysis in mixtures of sera and antigens, the suspicion was strong that complement was inactivated or absorbed in some manner rather than that the absence of hemolysis was due to the direct destructive action of serum and antigen upon complement.

As shown in Table 4, fresh, normal dog and rabbit sera may show well-marked inhibition of hemolysis with bacterial and lipoidal extracts, but, when this does not occur, heating the sera at temperatures as low as 40 to 45 C. for one-half hour increases the complement-inhibiting property. In this manner the apparent complement-fixing power of a serum is developed at a lower temperature than the antihemolytic power of the serum alone, altho the complement-inhibiting power of a serum is best developed by exposure to higher temperatures, as 55-60

TABLE 4

The Complement-fixing Power of Fresh Dog and Rabbit Sera Heated at Varying Temperatures for 30 Minutes

Temperature, C.	Cholesterinized Alcoholic Extract of Heart	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	B. coli	Serum Control	Cholesterinized Alcoholic Extract of Heart	Extract Acetone Insoluble Lipoids of Heart	Staphylo cocci	B. coli	Serum Control	Cholesterinized Alcoholic Extract of Heart	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	B. coli	Serum Control
	Dog Serum 539					Dog Serum 440					Dog Serum 329				
Fresh	—	—	—	—	—	—	—	++++	++++	—	++	++	++++	++++	—
40	—	—	+	+	—	±	—	++++	++++	—	++	++	++++	++++	—
45	—	—	+++	+++	—	±	—	++++	++++	—	++	++	++++	++++	±
50	+	+	+++	+++	—	+	—	++++	++++	—	++	++	++++	++++	++
55	++++	++++	++++	++++	—	++	+	++++	++++	—	++	++	++++	++++	—
60	++++	++++	++++	++++	+	++	+	++++	++++	—	—	—	++	++	—
62	+	+	++	++	—	+	±	++	+++	—	—	—	—	—	—
65	±	±	+	+	—	—	—	+	+	—	—	—	—	—	—
70	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—
80	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Rabbit Serum 23					Rabbit Serum 24					Rabbit Serum 65				
Fresh	—	—	—	—	—	—	—	—	—	—	+	—	++	++	—
40	—	—	—	—	—	—	—	—	—	—	+	—	++	++	—
45	—	—	—	—	—	—	—	—	—	—	++	±	+++	+++	—
50	++	±	+++	+++	—	—	—	—	—	—	++	++	++++	++++	—
55	++++	++++	++++	++++	—	++++	++	++++	++++	—	++++	++++	++++	++++	—
60	+++	++	++++	++++	—	++++	++	++++	++++	—	++++	++++	++++	++++	—
62	—	—	++	++	—	++	±	+	+	—	—	—	—	—	—
65	—	—	—	+	—	±	±	+	+	—	—	—	—	—	—
70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
80	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Key to Tables 4, 5, 6, 8, 10, and 11

++++ = complete inhibition of hemolysis (strongly positive).
+++ = 75% inhibition of hemolysis (moderately positive).
++ = 50% inhibition of hemolysis (weakly positive).
+ = 25% inhibition of hemolysis (very weakly positive).
± = delayed hemolysis or doubtful reaction).
— = complete hemolysis (negative reaction).

C., just as the serum antihemolytic substances are best developed at this temperature.

We have tested in this manner 12 dog and 6 rabbit sera, those shown in the tables being selected to show the irregularities and wide limits that were found. At 62 C. many sera, not all, lose their power of inhibiting complement activity with lipoidal, but not with bacterial, antigens; 65 C. exerts a greater influence, while practically all sera lose this property with all antigens when heated at 70 C. for one-half hour. As previously stated, the antihemolytic activity of a serum alone was likewise in our experiments invariably extinguished at this temperature.

As shown in Table 5, the complement-inhibiting power of dog and rabbit sera with lipoidal antigens became evident after an exposure of 10 or 20 minutes at 56 C. and much stronger after 30 and 45 minutes, while more prolonged exposure (1½ hours) did not tend to decrease this action. The results shown in this table are with sera selected among similar tests with 15 different dog, and 6 different rabbit sera, and they represent the results generally observed. As pointed out in our previous papers, rabbit sera show this complement-inhibiting power in the presence of various antigens to a much less degree than dog sera, the results in this respect being similar to the results in our study of the antihemolytic action alone of these sera.

Of interest in this connection is the relative thermostability of specific immune amboceptors and the non-specific antihemolytic and complement-inhibiting substances. For studying this relation we have selected the sera of mules immunized against glanders or suffering with that disease, also the sera of rabbits immunized with sheep cells and various bacteria and containing specific antibodies in addition to the non-specific complement-inhibiting substances. Mule sera were selected in addition to dog and rabbit sera because they behave very much as the latter in complement-fixation tests.

Heating these sera at 62 C. removed to a large degree, but not entirely, the non-specific complement-inhibiting substances with but slight depreciation of the immune amboceptors, as determined by titrating each serum in amounts ranging from 0.001 to 0.2 c.c. with constant doses of antigen; at 65 C. the non-specific reaction is still more influenced, while at 70 C. the non-specific reaction was not in evidence, altho in those sera containing large amounts of specific antibodies specific complement fixation occurred with the larger doses of serum.

TABLE 5

Complement Fixation with Normal Dog Serum (0.1 c.c.) and Normal Rabbit Serum (0.2 c.c.) Active and Heated at 56 C. for Varying Intervals of Time and with Various Lipoidal and Bacterial Antigens

Exposure, Min.	Cholesterinized Alcoholic Extract of Heart	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	B. coli	Serum Control	Cholesterinized Alcoholic Extract of Heart	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	B. coli	Serum Control	Cholesterinized Alcoholic Extract of Heart	Extract Acetone Insoluble Lipoids of Heart	Staphylococci	B. coli	Serum Control
	Serum 3 (Dog)					Serum 9 (Dog)					Serum 11 (Dog)				
Active	—	—	+++	+++	—	—	—	+	++	—	—	—	+	++	—
10	—	—	+++	+++	—	—	—	+	++	—	—	—	+	++	—
20	+	+	++++	++++	—	—	—	++	+++	—	—	—	+	++	—
30	++	+	++++	++++	±	++	—	++++	++++	—	—	—	+	++	—
45	+++	++	++++	++++	+	++++	+	++++	++++	—	±	±	+++	++++	—
60	++++	+++	++++	++++	+	++++	++	++++	++++	—	+	±	+++	++++	—
90	++++	++	++++	++++	+	++++	++	++++	++++	—	+	±	+++	++++	—
	Serum 41 (Rabbit)					Serum 30 (Rabbit)					Serum — (Rabbit)				
Active	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—
10	—	—	+	+	—	—	—	—	—	—	—	—	++	++	—
20	+	+	++	++	—	—	—	—	—	—	+	—	+++	+++	—
30	++	++	++++	++++	—	—	—	—	—	—	++	±	++++	++++	—
45	+++	+++	++++	++++	—	—	—	—	—	—	++	+	++++	++++	—
60	++++	+++	++++	++++	—	—	—	—	—	—	++	+	++++	++++	—
90	++	++	+++	++	—	—	—	—	—	—	+	+	++++	++++	—

Table 6 shows these results with the serum of a glanderous mule. While the specific glanders antibody withstood the higher temperatures in sufficient degree to absorb complement, whereas the non-specific substances were in large part, or entirely, removed, it is apparent that both are affected by the same temperatures, and the greater absorption of complement in the complement-fixation tests is due to the antilytic action of serum and antigen alone in addition to specific absorption by the antigen antibody combination. Of practical importance is the observation that heating a serum at 62 to 65 C. for one-half hour largely removes the non-specific factor without total destruction of the specific antibodies.

TABLE 6

COMPARATIVE THERMOSTABILITY OF SPECIFIC GLANDERS AMBOCEPTORS AND NON-SPECIFIC ANTILYTIC SUBSTANCES

Dose c.c.	Heated at 56 C.				Heated at 65 C.				Heated at 70 C.			
	Complement-Fixation Test*		Antilytic Test		Complement-Fixation Test		Antilytic Test		Complement-Fixation Test		Antilytic Test	
	I	II	I	II	I	II	I	II	I	II	I	II
0.001	+	—	—	—	—	—	—	—	—	—	—	—
0.005	+	—	—	—	+	—	—	—	—	—	—	—
0.01	++	—	—	—	++	—	—	—	±	—	—	—
0.05	+++	++	+	—	+++	+	—	—	++	—	—	—
0.1	++++	++++	+++	++	++++	+++	++	—	++	+	—	—
0.2	++++	++++	+++	++	++++	++++	++	+	+++	+++	—	—

* The complement-fixation tests were conducted with one quarter of the antilytic dose of a glanders antigen. For the sera of glanderous animals and glanders antigen I am indebted to Dr. Fred Boerner of the State Live Stock and Sanitary Board of Pennsylvania.

THE RELATION OF COMPLEMENTOIDS AND AMBOCEPTOIDS TO NON-SPECIFIC COMPLEMENT FIXATION

As previously stated, Ehrlich and Sachs found that at 60 C., but not at 50 C., dog serum becomes antihemolytic. According to their view, the zymotoxic group is destroyed by the higher temperatures and complementoid produced, so that in hemolytic tests the complementoid would be capable of union with amboceptors without exerting lytic activity and in this manner would block the action of complement. They also observed that the haptophoric group is weakened, since complementoid exerts a weaker combining power for amboceptor than the original complement. Neisser and Friedemann[13] sought to explain the phenomenon on the basis of the formation of amboceptoids. The experiments of Noguchi, however, have shown that the antilytic action of heated serum is due to anticomplementary activity, and our own experiments support this view.

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[13] Berl. klin. Wchnschr., 1902, 39, p. 677.

Six selected sera were heated at 56 C. for one-half hour. The antilytic activity of these was determined as was also their power of absorbing complement with the usual lipoidal and bacterial antigens. A portion of each serum was then treated with sheep cells which had been previously sensitized with 2 units of antisheep hemolysin and washed 3 times, and a third portion of each serum with ox cells which had been previously sensitized with 10 units of antiox hemolysin, and washed to remove unbound amboceptor. These mixtures were kept at room temperature for 2 hours to permit the union of complementoid to the sensitized corpuscles, by means of the haptophoric groups of the amboceptors. At the end of this time the corpuscles were separated by centrifugation and the supernatant fluid (diluted serum) tested in the same amounts for antilytic activity and complement-fixing power with antigens.

In 4 sera digestion with blood corpuscles was followed by partial removal of the antilytic principles, while in 2 sera a slight increase in antilytic activity was apparent; non-specific complement fixation was not influenced, so that if complementoids had been produced, either they were not attached to the hemolytic amboceptors, or, if complementoids are to be regarded as playing a rôle in the process, we must surmise not only the destruction of the zymotoxic group, but also destruction, or great weakening, of the haptophoric group so that complementoid was not attached to the blood cells.

The possible relationship of amboceptoids to the process of non-specific complement fixation was then studied experimentally. The majority of dog sera contain appreciable quantities of natural antisheep hemolysin. These amboceptors were removed from 6 selected sera by means of saturation with washed sheep cells and the sera tested for their antilytic and complement-fixing powers. Three of these extracted sera, when tested unheated after digestion with blood cells, were found to have yielded stronger complement-fixation; after heating the sera at 56 C. for 30 minutes their antilytic and complement-fixing powers were practically equal to those shown by portions of each serum heated and tested without previous removal of hemolytic amboceptors.

Portions of each serum were then heated at 56 C. for one-half hour and mixed with fresh, washed sheep and ox cells and placed in the refrigerator for 18 hours to permit the union of amboceptoid (hemolytic) to the cells by means of their cytophilic arms. At the end of this time the mixtures were centrifugated and the sera separated. These sera were then tested for their antilytic and complement-fixing powers. In 3 sera the antilytic activity was slightly increased, and in 3 other sera there was either removal or weakening of the antilytic and complement-fixing power. These results were similar to those shown in Tables 7 and 8.

Both these experiments were repeated with emulsions of Staphylococcus, B. coli, and B. typhosus, instead of emulsions of erythrocytes, with the object of dealing with any amboceptors for these that might be present. The results were identical with those observed with the corpuscles, and it is difficult to account for the antilytic and complement-fixing powers of these sera on the basis of amboceptoid formation.

TABLE 7

ANTILYTIC PRINCIPLES OF HEATED DOG SERUM SLIGHTLY REMOVED BY SENSITIZED BLOOD CELLS

Amount of Serum	Dog 42 (56 C.)		Dog 80 (56 C.)		Dog 96 (56 C.)		Dog 142 (56 C.)	
	Before Digestion	After Digestion	Before Digestion	After Digestion	Before Digestion	After Digestion	Before Digestion	After Digestion
0.005	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.01	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.05	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Trace	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.1	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.15	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Moderate hemolysis (slightly antilytic)	Moderate hemolysis (slightly antilytic)	Complete hemolysis	Complete hemolysis
0.2	Moderate hemolysis (slightly antilytic)	Marked hemolysis	Complete hemolysis	Moderate hemolysis (slightly antilytic)	None	None	Moderate hemolysis (slightly antilytic)	Marked hemolysis

REGARDING ABSORPTION OF THE ANTILYTIC AND COMPLEMENT-FIXING SUBSTANCES OF DOG SERUM BY BLOOD CORPUSCLES

According to Noguchi, corpuscles of various animals can deprive heated sera of their antilytic principles, the corpuscles becoming more resistant to serum hemolysis. As stated above, when we digested heated sera with washed sheep or ox cells, the antilytic activity of the

TABLE 8

NON-SPECIFIC COMPLEMENT-ABSORBING SUBSTANCES OF HEATED DOG SERUM NOT REMOVED BY SENSITIZED BLOOD CELLS (SERUM HEATED AT 56 C. DOSE 0.2 c.c.)

Dog	Before Digestion							After Digestion						
	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	B. coli	Staphylococci	B. typhosus	Serum Control	Cholesterinized Alcoholic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Extract Acetone Insoluble Lipoids of Heart	B. coli	Staphylococci	B. typhosus	Serum Control
42..........	+	−	−	++	++	++	+	+	−	−	+++	++	++	±
80..........	++	+	+	+++	+++	+++	−	++	+	+	++++	++++	++++	+
96..........	+++	++	++	++++	++++	++++	++	+++	++	++	++++	++++	++++	++
142..........	+++	++	++	++++	++++	++++	+	+++	++	++	++++	++++	++++	+

sera was increased in some instances and decreased in others. The complement-fixing powers of the sera with the various antigens employed were not influenced. But when the sheep and ox cells used in treating the heated sera were removed from the sera after 18 hours in the refrigerator, washed 3 times, and re-suspended in normal salt solution in a 2.5% suspension and compared with a similar suspension of untreated cells, preserved in the refrigerator for the same length of time, no appreciable differences in the susceptibility of the cells to lysis by the same doses of amboceptors and complement could be detected. In Table 9 are shown 4 titrations of sheep cells used in digesting the 4 heated dog sera shown in Tables 7 and 8.

TABLE 9

SHEEP CORPUSCLES DO NOT BECOME MORE RESISTANT TO SERUM HEMOLYSIS AS A RESULT OF DIGESTION WITH HEATED DOG SERUM (ANTISHEEP HEMOLYSIN IN DOSE OF 0.005 C.C. WITH 0.05 C.C. MIXED GUINEA-PIG SERUM COMPLEMENT)

Amount of Cells, c.c.	Dog 42		Dog 80		Dog 96		Dog 142	
	Cells Before Digestion	Cells After Digestion	Cells Before Digestion	Cells After Digestion	Cells Before Digestion	Cells After Digestion	Cells Before Digestion	Cells After Digestion
0.05	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.1	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.3	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.5	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
0.8	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
1.0	Moderate hemolysis (slightly hemolytic)	Moderate hemolysis (slightly hemolytic)	Moderate hemolysis (slightly hemolytic)	Moderate hemolysis (slightly hemolytic)	Moderate hemolysis (slightly hemolytic)	Moderate hemolysis (slightly hemolytic)	Moderate hemolysis (slightly hemolytic)	Moderate hemolysis (slightly hemolytic)

According to our experiments, therefore, blood corpuscles act indifferently in the absorption of the antilytic and complement-fixing principles of dog serum; frequently the reverse was observed, the antilytic activity of the serum being increased. Similar results were observed in the following set of experiments.

RELATION OF BACTERIOLYTIC AMBOCEPTORS TO NON-SPECIFIC COMPLEMENT FIXATION BY DOG AND RABBIT SERA

Because of the fact that a large percentage of normal dog and rabbit sera absorb complement to a well-marked degree in the presence of various bacterial antigens, as pointed out in our previous papers, it was necessary to learn whether bacteriolytic amboceptors were present, altho the reactions gave indications of being non-specific.

We have not tested the bacteriolytic power of serum, but have studied the question by means of absorption tests. Six dog, and 9 rabbit sera were heated at 56 C. for one-half hour and used in complement-fixation tests in dose of 0.2 c.c. with lipoidal and 3 bacterial antigens (Staphylococcus, B. coli, and B. typhosus). Two cubic centimeters of each serum were then absorbed for 18 hours with 0.2 c.c. of a thick, washed emulsion of each of the three bacterial cultures. At the end of this time the mixtures were subjected to prolonged centrifugation at high speed and the clear sera separated from the sediment of bacteria and tested in the same manner with the same antigens.

As shown in Table 10, absorption with these bacteria tends to remove a portion of the principles in the sera responsible for the fixation of complement with these antigens. On the other hand, absorption appeared to increase the antilytic power of some sera, as well as the degree of complement fixation with the antigens, acting in both these respects in a manner similar to that of absorption with blood corpuscles. Likewise, the absence of specificity is evidence against the rôle of specific amboceptors or amboceptoids in the process, as digestion of serum with staphylococci absorbs a portion of the principles reacting with such antigens as alcoholic extract of heart, B. coli, and B. typhosus, as well as with Staphylococcus; similar results were observed after digestion with B. coli and B. typhosus . It would appear therefore, that the antilytic and complement-fixing principles are non-specific and capable of partial absorption, not only by blood corpuscles, but by bacterial cells as well.

THE RELATION OF PARASITES TO NON-SPECIFIC COMPLEMENT FIXATION BY NORMAL DOG AND RABBIT SERA

Coccidiosis in rabbits has been regarded by a few investigators as a probable factor in the phenomenon of non-specific complement fixation. Among the animals in our previous study[14] and likewise among those of the present series, coccidiosis was relatively frequent, but we have not been able to trace any relation between this disease and the property in the sera of non-specific complement fixation—animals

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[14] Kolmer and Casselman: Jour. Med. Research, 1913, 28, p. 369.

TABLE 10

Influence of Absorption by Bacteria on the Antilytic and Complement-fixing Powders of Dog and Rabbit Sera

Serum 56 C.	Before Absorption					After Absorption with Cocci					After Absorption with B. coli					After Absorption with B. typhosus				
	Cholesterinized Alcoholic Extract of Heart	Staphylococci	B. coli	B. typhosus	Serum Control	Cholesterinized Alcoholic Extract of Heart	Staphylococci	B. coli	B. typhosus	Serum Control	Cholesterinized Alcoholic Extract of Heart	Staphylococci	B. coli	B. typhosus	Serum Control	Cholesterinized Alcoholic Extract of Heart	Staphylococci	B. coli	B. typhosus	Serum Control
Dog 1...	++	++++	++	++++	+	++	++++	++	++++	++	++	++++	++	++++	+	++	++++	++	++++	+
Dog 2...	+	++	++	++	—	+	+++	+++	+++	—	+	++	+++	+++	—	+	++	+++	+++	—
Dog 3...	++++	++++	++++	++++	++	++	++++	++	++	++	++	++++	++	++	++	++	++++	++	++	++
Dog 4...	++++	++++	++++	++++	+	+	++++	++	++	+	+	++++	++	++	+	+	++++	++	++	+
Dog 5...	++++	++++	++++	++++	++	+	++	+	+	+	+	++	+	+	+	+	++	+	+	+
Dog 6...	++++	++++	++++	++++	++	—	+++	—	—	—	—	+++	—	—	—	—	+++	—	—	—
Rabbit 1	—	++++	+	++	—	++	+++	++	++	++	++	+++	++	++	++	++	+++	++	++	+
Rabbit 2	±	++++	+	+	—	+	+++	++	++	+	+	+++	++	++	+	+	+++	++	++	+
Rabbit 3	—	++	—	—	—	—	+	—	—	—	—	—	+	—	—	—	—	+	—	—
Rabbit 4	++++	++++	++++	++++	—	—	++	—	—	—	—	++	—	—	—	—	++	—	—	—
Rabbit 5	—	+++	—	—	—	—	++	—	—	—	—	—	++	—	—	—	—	++	—	—
Rabbit 6	—	+++	—	—	—	—	+	—	—	—	—	—	+	—	—	—	—	+	—	—

with advanced lesions have reacted negatively, and young rabbits with no evidences of the infection have reacted positively.

Many of the dogs used in these studies have been examined post mortem in the hope of finding some lesion or some parasitic infection that might explain the positive reaction. Parasites, as for example Cysticercus pisiformis and Coccidium cuniculi, have frequently been found, but these appear to bear no relation to the occurrence of a positive reaction.

We have paid particular attention to the probable relation of intestinal parasites in dogs to non-specific complement fixation and to the question of specific complement fixation in intestinal parasitism (the results of these studies are given elsewhere), but we do not believe that these parasites are directly concerned in producing the principles in the serum responsible for the antilytic properties of dog serum and its tendency to absorb complement in a non-specific manner with lipoidal tissue extracts and bacterial antigens.

THE INFLUENCE OF SALVARSAN UPON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM *

Emanuel[15] claims that the administration of salvarsan tends to remove the property in rabbit serum of absorbing complement in a non-specific manner. Epstein and Pribram[16] have made a similar claim for mercury.

In the past year, during chemotherapeutic researches, we have used rabbits for determining the lethal dose of salvarsan, and have performed complement-fixation tests with their sera before, and at varying intervals after, the intravenous administration of 70 milligrams per kilogram of body weight. This dose represents 4.2 grams for a man weighing 60 kilos, and is the maximal dose of the usual preparation of salvarsan that may be administered to rabbits with safety. The results are shown in Table 11. Occasionally a slight weakening in the reactions with inactivated serum was noted, but, as a general rule, the administration of a single large dose of salvarsan was without effect upon the property of normal rabbit serum for absorbing complement in a non-specific manner.

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* From the Department of Dermatological Research, Philadelphia Polyclinic and College for Graduates in Medicine.

[15] Berl. klin. Wchnschr., 1911, 48, p. 2335.

[16] Ztschr. f. exper. Path. u. Therap., 1909, 7, p. 549.

TABLE 11

THE INFLUENCE OF SALVARSAN UPON NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT SERUM

No.	Salvarsan (Intravenous Injection)	Active Serum (0.2 c.c.)					Inactivated Serum (0.2 c.c.)				
		Choles-terinized Alco-holic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Staph-ylo-cocci	B. coli	Serum Con-trol	Choles-terinized Alco-holic Extract of Heart	Alcoholic Extract of Syphilitic Liver	Staph-ylo-cocci	B. coli	Serum Con-trol
44	Before salvarsan	—	—	++++	++++	—	+	+	++++	++++	—
44	36 hr. after 70 mg. per kilo	—	—	++	++	—	+	+	++++	++++	—
44	14 days later	—	—	++++	++++	—	++	++	++++	++++	—
44	21 days later	—	—	++	++	—	++	++	++++	++++	—
33	Before salvarsan	++++	++++	++++	++++	—	++++	++++	++++	++++	—
33	4 days after 70 mg. per kilo	—	—	—	—	—	++++	++++	++++	++++	—
33	12 days later	—	—	—	—	—	++++	++++	++++	++++	—
33	28 days later	—	—	—	—	—	++++	++++	++++	++++	—
29	Before salvarsan	—	—	—	—	—	++	++	++++	++++	—
29	48 hr. after 100 mg. per kilo	—	—	—	—	—	+	+	++++	++++	—
29	28 days later	—	—	—	—	—	++	++	++++	++++	—
29	40 days later	—	—	—	—	—	+++	+++	++++	++++	—
29	45 days later	—	—	—	—	—	+++	+++	++++	++++	—
37	Before salvarsan	—	—	—	—	—	+	+	++	++	—
37	Immediately after 70 mg. per kilo	—	—	—	—	—	+	+	++	++	—
37	24 hr. later	—	—	—	—	—	++	++	++	++	—
60	Before salvarsan	—	—	—	—	—	++++	++++	++++	++++	—
60	24 hr. after 70 mg. per kilo	—	—	—	—	—	++++	++++	++++	++++	—
60	5 days later	—	—	—	—	—	++	++	++++	++++	—
62	Before salvarsan	—	—	—	—	—	++++	++++	++++	++++	—
62	24 hr. after 70 mg. per kilo	—	—	—	—	—	++++	++++	++++	++++	—
62	5 days later	—	—	—	—	—	++	++	++++	++++	—
62	49 days later	—	—	—	—	—	++	++	++++	++++	—
27	Before salvarsan	—	—	—	—	—	++	+	+++	+++	—
27	Immediately after 70 mg. per kilo	—	—	—	—	—	++	+	+++	+++	—
28	Before salvarsan	—	—	—	—	—	++	+	++	++	—
28	24 hr. after 70 mg. per kilo	—	—	—	—	—	+++	++	+++	+++	—
28	96 hr. later	—	—	—	—	—	++	+	+++	+++	—

Mercury likewise appears to have no influence on this property of rabbit sera. We have administered daily intramuscular injections of soluble salts over a period of several weeks without definite influence on the serum reactions.

QUANTITATIVE FACTORS IN NON-SPECIFIC COMPLEMENT FIXATION WITH NORMAL RABBIT AND DOG SERA

As in all complement-fixation tests, quantitative factors have a direct and considerable influence on the results. In this and previous papers we have emphasized the importance of quantitative factors in non-specific complement fixation with rabbit and dog sera. As is well known, an excess of antigen, a decrease of complement, and a serum containing thermostabil anticomplementary bodies are factors which may produce false positive reactions with any serum; conversely, an excess of complement or hemolysin tends to mask absorption or fixation of complement, yielding false negative reactions.

In all our work the lipoidal extracts were used in doses equal to 6 to 12 times less than their anticomplementary doses, and the bacterial emulsions in one-quarter of their anticomplementary doses. The use of larger doses tended to increase the percentage of non-specific reactions, and smaller doses yielded fewer reactions.

As the serum complements of guinea-pigs vary in their adaptability for fixation, the mixed sera of at least 2 pigs were generally used in dose of 0.05 c.c. (1 c.c. of a 1:20 dilution). This dose of complement has been found reliable and was used as a fixed unit in titrating the hemolysin against a fixed dose of corpuscle suspension (1 c.c. of a 2.5% suspension of washed sheep cells). The hemolysin was used in an amount equal to double the unit, being titrated each day with the complement serum and corpuscles. The use of a smaller dose of hemolysin increases the tendency for non-specific reactions, and the so-called "single unit" system should never be used in conducting complement-fixation tests with rabbit and dog sera.

With the technic employed by us the amount of complement absorbed was rather variable with the sera of different animals. When Kolmer's method for determining the degree of complement fixation according to the number of units of hemolytic complement absorbed, was used, of the lipoidal extracts most absorption occurred with the alcoholic extract of heart re-enforced with cholesterin, and with bacterial antigens more complement was usually absorbed than with the lipoidal extracts. As a general rule, dog serum absorbed more complement than did rabbit serum, and in all instances heated serum more than unheated. Even with sera yielding strong reactions according to the usual technic, the amount of complement absorbed according to the quantitative method was relatively small and generally much less than that absorbed by the serum of a syphilitic person with the ordinary lipoidal extracts.

A large proportion of fresh rabbit sera contain natural or native antisheep hemolysin. Kolmer and Williams[17] found that 36% of rabbit sera in doses of 0.2 c.c. contain sufficient antisheep hemolysin to cause complete lysis of 1 c.c. of a 2.5% suspension of cells with 0.05 c.c. complement, the same quantities used in the complement-fixation tests of this work. Likewise, a large proportion of normal dog sera contain antisheep hemolysin, and usually 0.2 c.c. of fresh dog serum contains sufficient hemolysin and complement to cause complete lysis of 1 c.c. of a 2.5% suspension of sheep cells.

The presence of these natural amboceptors in addition to complement in fresh unheated serum probably explains in part the smaller percentage of non-specific reactions when active serum is used, slight degrees of complement absorption being masked.

With sera, however, in which the hemolytic complement and antisheep hemolysin are titrated and known, the well-defined absorption or fixation of complement that follows with sera heated at 55 C. for one-half hour, as compared with unheated sera, cannot be entirely explained on the basis that the native complement has been inactivated and native hemolysin possibly weakened; on the contrary, some chemical change has probably occurred in the nature of an alteration of the colloidal conditions, so that in a mixture of serum, complement, and antigen, complement is absorbed in a non-specific manner.

It is to be emphasized that in conducting complement-fixation tests with dog and rabbit sera, the antigen should be carefully titrated, and not used in an amount over one-quarter of its anticomplementary dose; if the serum is used after inactivation, the dose of complement should be sufficiently large, and the immune hemolysin should be used in an amount equal to at least double its hemolytic unit.

DISCUSSION OF THE NATURE OF NON-SPECIFIC COMPLEMENT FIXATION BY NORMAL RABBIT AND DOG SERA

While it is not possible to make definite statements, on the basis of experimental evidence, regarding the nature of the substances in serum responsible for non-specific complement fixation, it is highly probable that these substances are similar to, or identical with, the antilytic substances. The slight differences may be only apparent, and just as a human serum may show complete hemolysis in a serum control-tube of a complement-fixation test and yet be slightly anticomplementary, yielding well-marked non-specific inhibition of hemolysis in the presence of the antigen, so in like manner the complement-absorbing properties of dog and rabbit sera in complement-fixation tests stand out more prominently than the purely antilytic powers.

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[17] Jour. of Med. Research, 1913, 28, p. 369.

In our opinion, the phenomenon is entirely non-specific, the complement fixation being due to the antilytic substances plus the antilytic properties of even minute amounts of the extracts and emulsions used as antigens.

Objections may be raised therefore, against calling this process non-specific complement fixation. We have purposely adhered to this terminology because the phenomenon may be detected only in complement-fixation tests and it is probable that the antilytic substances may, with the antigen, truly absorb, or fix, complement. While the antilytic substances are now believed to be anticomplementary, the exact influence upon complements is not known.

In complement-fixation tests with dog and rabbit sera the antilytic tendency of the serum may not be in evidence because of ready and complete hemolysis of the serum control-tubes, and yet some degree of inhibition of hemolysis may result in those tubes containing antigen. We have sought to emphasize the importance of these non-specific reactions because of their bearing upon practical and experimental work with these sera, in addition to the light that may be thrown on the mechanism of complement fixation in general, and upon the Wassermann reaction in particular.

CONCLUSIONS

1. Non-specific complement fixation by normal rabbit and dog sera is probably due primarily to thermolabil and thermostabil antilytic (anticomplementary) substances in the sera.

2. While fresh and active rabbit and dog sera may yield non-specific complement-fixation, the tendency is greatly increased as a result of heating the sera. At 56 C. the changes may occur in 20 minutes or even less; at 62 C. for 30 minutes the tendency for non-specific reactions is much decreased, and is entirely removed by heating

serum at 70 C. for 30 minutes. Changes may occur after exposure at 45 C. for 30 minutes, but the optimal temperature is between 55 and 60 C.

3. In complement-fixation tests for specific antibodies with inactivated rabbit, dog, and mule sera, it is advisable to heat the sera at 62 C. for one-half hour and to use at least 2 units of complement or hemolysin and no more than one-quarter of the anticomplementary unit of antigen after it has been carefully titrated.

4. Complementoids and amboceptoids probably bear no relation to the process of non-specific complement fixation by rabbit and dog sera.

5. The blood corpuscles of various animals and various bacteria may absorb a portion of the antilytic substances from rabbit and dog sera, but they have much less influence on the complement-fixation reactions. Digestion of fresh sera with corpuscles and bacteria not infrequently increases the anticomplementary properties of the sera.

6. Bacteriolytic amboceptors are not responsible for non-specific complement fixation by normal rabbit and dog sera.

7. Parasitic infestations of rabbits and dogs bear no relation to the antilytic and complement-fixing properties of the sera.

8. Single, large doses of salvarsan are without definite influence on the reactions with rabbit serum.

9. Quantitative factors in the hemolytic system and antigen are of considerable importance in relation to these non-specific reactions.

10. If time permits, preliminary complement-fixation tests should be performed with the sera of rabbits or dogs before immunization or inoculation is begun, and only those animals selected the sera of which react negatively with the antigen used.